ANNALS OF CARNEGIE MUSEUM
VOL. 59, NUMBER 3, PP. 249–259 5 SEPTEMBER 1990

GENIC RELATIONSHIPS AMONG NORTH AMERICAN *MICROTUS* (MAMMALIA: RODENTIA)

DWIGHT W. MOORE[1]

LAURA L. JANECEK
Postdoctoral Fellow, Section of Mammals

ABSTRACT

Relationships among nine species of *Microtus* from North America were examined using starch-gel electrophoresis. *Clethrionomys gapperi* served as an outgroup in the phenetic analyses. An unrooted tree produced by a Fitch-Margoliash analysis indicated that *M. oregoni* and *M. longicaudus* are genically distinct from other North American species of *Microtus*; both taxa occupy branches separate from the other species examined. *Microtus pennsylvanicus* is most similar to *M. montanus*; these taxa then clustered as most similar to *M. mexicanus*. *Microtus ochrogaster* is most similar to *M. quasiater*, corroborating previous analyses of dental characters that suggested that these two taxa are closely related. *Microtus pinetorum* clusters with *M. californicus*, rather than with other taxa considered by some investigators to be North American representatives of the genus *Pitymys*. Analyses of allozymic variation produced no evidence documenting the separation of the nominal taxa *M. pinetorum*, *M. ochrogaster*, and *M. quasiater*, North American taxa often allocated to the genus *Pitymys*, from other North American taxa of *Microtus*. We conclude that the genus *Pitymys*, as currently constituted, is polyphyletic.

INTRODUCTION

The genus *Microtus* (*sensu lato*) occurs throughout North America, Europe, and much of Asia. Including the species of *Pitymys*, there are approximately 70 extant species worldwide; 23 of these species occur in North America (Anderson, 1985). Repenning (1983) examined tooth morphology of fossil and extant species and postulated that *Microtus* and *Pitymys* shared a common ancestor, *Allophaiomys*, approximately 1.2 million years ago. Since that time *Microtus* and *Pitymys* have diverged and supposedly represent separate monophyletic lineages that are sufficiently distinct from one another to warrant being placed in separate genera. Kretzoi (1969), Van der Meulen (1978), and Zakrzewski (1985) shared this opinion. However, Anderson (1985), Dalquest et al. (1969), Hall (1981), and Hooper and Hart (1962) believed that *Pitymys* was not sufficiently distinct from *Microtus* to warrant giving it separate generic status. These authors included *Pitymys* as a subgenus within *Microtus*. Primarily, these systematic conclusions were based upon cranial characters (Dalquest et al., 1969), morphology of the molars (Repenning, 1983; Van der Meulen, 1978), or a combination of cranial and penile morphology (Hooper and Hart, 1962). In addition, it is unclear which North American species to include in *Pitymys*. Hall (1981), following Hooper and Hart (1962), included only *M. pinetorum* and *M. quasiater* in the subgenus *Pitymys* and believed that *M. mexicanus* and *M. ochrogaster* were closely related but placed them in the subgenus *Microtus*. Repenning (1983) included the nominal taxa *P. pinetorum*, *P. ochrogaster*, *P. nemoralis*, and *P. quasiater* in the genus *Pitymys*.

[1] Division of Biological Sciences and Schmidt Museum of Natural History, Emporia State University, Emporia, KS 66801.
Date submitted: 2 February 1990.

The relationships among members of the genus *Microtus,* exclusive of those assigned to *Pitymys,* are also unclear. Anderson (1985) summarized, as cladograms, the proposed classifications of Chaline (1980) and Hooper and Musser (1964) that concerned the evolutionary relationships among *Microtus.* Specifically, Chaline (1980) proposed an association among *M. longicaudus, M. californicus, M. montanus,* and *M. mexicanus,* based upon examination of dental characters. Chaline (1980) proposed that each of the remaining taxa of *Microtus* examined (*M. pennsylvanicus, M. pinetorum, M. ochrogaster,* and *M. oregoni*) was distinct from other taxa and constituted a separate branch on the cladogram (Anderson, 1985). Hooper and Musser (1964) found *M. montanus* to be most similar to *M. pennsylvanicus,* based upon characteristics of the glans penis; other taxa of *Microtus* were not grouped into any obvious associations. Graf (1982) presented a phenogram in which *M. californicus* was genically most similar to *M. pinetorum,* whereas *M. ochrogaster* was genically most similar to *M. montanus.* Modi (1987) examined chromosomal banding patterns among Nearctic voles and proposed that *M. pennsylvanicus* was most closely related to five other *Microtus* taxa, including *M. oregoni* and *M. montanus. Microtus ochrogaster* was tentatively placed with *M. pinetorum,* although this association was based upon questionable karyotypic characters (Modi, 1987). *Microtus longicaudus* was a chromosomally distinct lineage, whereas the primitive karyotypes of *M. mexicanus* and *M. californicus* precluded taxonomic assessment of these taxa (Modi, 1987).

Previous studies of allozyme variation among *Microtus* from North America have included no more than three species (Nadler et al., 1978), except the study of Graf (1982), in which four or fewer individuals from single populations of each species were examined. In addition, no genic analysis has specifically addressed the validity of *Pitymys* as a lineage separate from *Microtus* in North America, although Chaline (1980) and Chaline and Graf (1988) found *M. pinetorum* to cluster phenetically with other species of *Microtus* rather than with European species of *Pitymys.*

The purpose of this study was to use genic data to analyze the evolutionary relationships among species of North American *Microtus* (*sensu lato*) and specifically to examine the relationship between North American *Microtus* and *Pitymys.* Nine species of *Microtus* from North American were examined. All the extant North American species that were placed in *Pitymys* by Repenning (1983) were included in this study so that the systematic status of *Pitymys* could be evaluated.

Materials and Methods

All specimens were captured in Sherman live traps. Heart, kidney, and liver samples were taken from each specimen immediately after death and were frozen in liquid nitrogen. Tissues were maintained at −60°C until processed. Heart and kidney extracts were processed together. Techniques of tissue preparation, horizontal starch-gel electrophoresis, and biochemical staining were similar to those described by Selander et al. (1971) or Harris and Hopkinson (1976). All gels were prepared using a 50:50 mixture of electrostarch lot 392 (Electrostarch Co., Madison, WI) and Sigma starch (Sigma Chemical Co., St. Louis, MO). In multiple locus systems, the isozyme migrating most anodally was designated as "1." Peptidase loci were designated for their substrate specificity. Alleles were designated alphabetically with the most anodally migrating allele designated as "A." All other alleles were assigned a letter in descending order from most anodal to most cathodal. The 25 presumptive loci examined (including Enzyme Commission numbers), included general protein-1, -2 (GP-1, -2), superoxide dismutase (SOD; 1.15.1.1), glycyl-leucine peptidase (P-GL; 3.4.11), leucyl-glycyl-glycine peptidase (P-LGG; 3.4.11), glucose phosphate isomerase (GPI; 5.3.1.9), sorbitol dehydrogenase (SDH; 1.1.1.14), aspartate aminotransferase-1, -2 (AAT-1, -2; 2.6.1.1), leucine amino peptidase (LAP; 3.4.11), glycerol-3-phosphate dehydrogenase (GPD; 1.1.1.8), glutamate dehydrogenase (GDH; 1.4.1.2), glucose-6-phos-

phate dehydrogenase (G6P; 1.1.1.49), alcohol dehydrogenase (ADH; 1.1.1.1), phosphoglucomutase-1, -2 (PGM-1, -2; 2.7.5.1), purine nucleoside phosphorylase (NP; 2.4.2.1), hexokinase (HK; 2.7.1.1), isocitrate dehydrogenase-1, -2 (IDH-1, -2; 1.1.1.42), lactate dehydrogenase-1, -2 (LDH-1, -2; 1.1.1.27), malate dehydrogenase-1, -2 (MDH-1, -2; 1.1.1.37), and malic enzyme (ME; 1.1.1.40).

When possible, tissues from ten individuals from a single locality were included for each species. For some species this was not possible, but all species were represented by at least three individuals. Gorman and Renzi (1979) proposed that three individuals often are sufficient for the determination of relationships among species. Specimens of *Clethrionomys gapperi* were included as a potential outgroup to the North American species of *Microtus* included in this study.

Coefficients of Rogers' (1972) genetic distance (*D*) were computed for all possible paired combinations from the allele frequency data for each population of each species. A phenogram for all 15 populations representing ten species was obtained from the distance matrix using the unweighted pair group method with arithmetic averages option of the BIOSYS-1 package of Swofford and Selander (1981). Phenetic relationships among taxa were further summarized in the form of an unrooted tree produced by a Fitch and Margoliash (1967) analysis of the Rogers' distance matrix using the PHYLIP package of Felsenstein (1989).

All specimens were prepared as skins with skeletons or were preserved in 10% formalin and transferred to 70% ethyl alcohol following removal of skulls. Specimens are deposited in the University of New Mexico Museum of Southwestern Biology unless indicated otherwise. Species designation of populations follows Hall (1981). Numbers in parentheses indicate sample sizes.

SPECIMENS EXAMINED

Clethrionomys gapperi—PENNSYLVANIA: Warren Co.; 1 mi S, 10 mi E Warren (5). *Microtus californicus*—CALIFORNIA: San Bernardino Co.; 10 mi SE Big Bear City (10). Sonoma Co.; 1.6 km S, 2.5 km W Bodega Bay (10). *Microtus longicaudus*—NEW MEXICO: Catron Co.; 12 mi E Mogollon (10). Taos Co.; 4 mi N, 11 mi E Arroyo Hondo (10). *Microtus mexicanus*—NEW MEXICO: Torrance Co.; 1.7 mi S, 4.6 mi W Manzano (10). Valencia Co.; 5.6 mi S, 14.9 mi W Grants (10). *Microtus montanus*—NEW MEXICO: Sandoval Co.; 3 mi N, 10.5 mi E Jemez Springs (4), 15 mi N, 2 mi E Jemez Springs (2). *Microtus ochrogaster*—ARKANSAS: Lonoke Co.; 0.5 mi N, 9.1 mi W Lonoke (10). MISSOURI: Platte Co.; 0.5 mi N, 2.1 mi E Parkville (4). *Microtus oregoni*—WASHINGTON: Clallam Co.; 9.2 mi S, 2.7 mi W Port Angeles (3). *Microtus pennsylvanicus*—MASSACHUSETTS: Franklin Co.; 1.3 mi W South Deerfield Center (10). *Microtus pinetorum*—ARKANSAS: Pulaski Co.; Little Rock (2). Saline Co.; 1 mi N, 2 mi W Bryant (3). MASSACHUSETTS: Franklin Co.; 0.3 mi N, 0.2 mi W Whately Center (3). *Microtus quasiater*—MEXICO. VERACRUZ: 2 km S (by road) Cuautlapan (2; Museum of Vertebrate Zoology, Berkeley); 4 mi N Jalapa (1).

RESULTS

Twenty-four loci were polymorphic for the ten species of microtines examined (Table 1). One locus (IDH-1) that was monomorphic across all species was not included in Table 1 but was used in calculating coefficients of genetic distance. In *Clethrionomys gapperi* eight loci were fixed for a different allele than was found in any species of *Microtus*. *Microtus mexicanus* and *M. pennsylvanicus* were each fixed for three unique alleles not found in any other taxon of *Microtus*. Intraspecific genic variation included fixed differences at two loci (SOD and HK) between populations of *M. californicus*, and a fixed difference at a single locus (G6P) between populations of *M. pinetorum*.

Coefficients of Rogers' (1972) genetic distance (*D*) were calculated for the 15 populations examined (Table 2). Mean intraspecific genetic distances between populations ranged from 0.063 in *M. ochrogaster* to 0.104 in *M. californicus*. Phenetic relationships based upon *D* values among the 15 populations examined (Fig. 1) indicated that North American *Microtus* separate genically into three groups: (1) populations representing *M. ochrogaster, M. quasiater, M. californicus, M. pinetorum, M. oregoni,* and *M. longicaudus,* (2) *M. montanus* and *M. pennsylvanicus,* and (3) *M. mexicanus*.

Phenetic relationships among populations were further summarized in the form of an unrooted Fitch and Margoliash (1967) tree (Fig. 2), in which branch lengths

Table 1.—*Allele frequencies at 24 variable loci for the populations of* Microtus *and* Clethrionomys gapperi *examined. The most anodal allele at a locus is designated "A." Unless otherwise indicated, alleles were present in a population at a frequency of 1.00.*

Species and populations	*n*	Loci											
		ME	GDH	MDH-1	MDH-2	LDH-1	LDH-2	ADH	PGM-1	PGM-2	G6P	SOD	P-GL
1. *M. longicaudus* Taos Co., NM	10	B/0.05 D/0.95	B/0.80 C/0.20	A	B	A	B	D	C	B	A/0.05 B/0.95	C	A
2. *M. longicaudus* Catron Co., NM	10	B/0.30 D/0.70	B	A	B	A	B	D	B/0.05 C/0.95	A/0.15 B/0.85	B	C	A
3. *M. mexicanus* Valencia Co., NM	10	C	B	A	B	A	A/0.20 B/0.80	E	C/0.45 E/0.40 G/0.15	B	C	C	A
4. *M. mexicanus* Torrance Co., NM	10	C	A/0.10 B/0.90	A	B	A	B	E	E	A/0.05 B/0.95	C	C	A
5. *M. ochrogaster* Lonoke Co., AR	10	B/0.10 C/0.65 D/0.25	B	A	B	A	B	A/0.40 C/0.05 D/0.55	C	B	B	C	A
6. *M. ochrogaster* Platte Co., MO	4	C	B	A	B	A	B	A/0.25 D/0.75	C	B	B	C	A
7. *M. oregoni* Clallam Co., WA	3	C	B	A	B	A	B	C/0.33 D/0.67	F	A/0.17 B/0.83	B	C	B
8. *M. montanus* Sandoval Co., NM	6	C	B	A	B	A	B	C	E	B	C	C	A
9. *M. pennsylvanicus* Franklin Co., MA	10	A	B	A	A	A	B	C/0.05 D/0.95	B/0.05 E/0.95	A/0.05 B/0.95	C	C	A
10. *M. californicus* San Bernardino Co., CA	10	C	C	A	B	A	B	C	C	B	B	C	A
11. *M. californicus* Sonoma Co., CA	10	C	C	A	B	A/0.90 C/0.10	B	C	C	B/0.95 C/0.05	B	A	A
12. *M. pinetorum* Franklin Co., MA	3	C	B	A	B	A	B	C	C	B	B	C	B
13. *M. pinetorum* AR	5	C/0.80 D/0.20	B	A	B	A	B	C	C	B	C	C	B
14. *M. quasiater* Veracruz, Mex.	3	C/0.83 E/0.17	B	A	B	A	B	B/0.33 C/0.67	A	B	B	C	A
15. *Clethrionomys* Warren Co., PA	5	F	B	B	B	A/0.80 B/0.20	B	C	D	B/0.90 D/0.10	B	B	B

Table 1.—*Continued.*

Species and populations	Loci											
	GPI	SDH	GPD	LAP	AAT-1	AAT-2	GP-1	GP-2	P-LGG	IDH-2	HK	NP
1. *M. longicaudus* Taos Co., NM	A	C/0.80 D/0.20	B/0.40 C/0.55 D/0.05	C	D	B/0.80 C/0.20	A	A	B	A	C	B/0.80 D/0.20
2. *M. longicaudus* Catron Co., NM	A	C	B/0.20 C/0.60 D/0.20	C	D	B/0.10 C/0.90	A	A	B/0.85 D/0.15	A	C	B/0.65 D/0.35
3. *M. mexicanus* Valencia Co., NM	A	A	B/0.05 C/0.95	C	A/0.25 D/0.75	C	A	B	A	A	C	A/0.80 E/0.20
4. *M. mexicanus* Torrance Co., NM	A/0.30 C/0.70	A	C	C	D	A/0.10 C/0.90	A	B	A	A	C	A
5. *M. ochrogaster* Lonoke Co., AR	C	C	C	C	A/0.30 D/0.60 E/0.10	C	A	A	A	A/0.40 B/0.60	C	B
6. *M. ochrogaster* Platte Co., MO	C/0.88 E/0.12	C	C	C	D	C	A	A	A	A	C	B
7. *M. oregoni* Clallam Co., WA	F	C	C	C	A	C	A	A	A	A	C	B
8. *M. montanus* Sandoval Co., NM	D	C	C	B	D	C	A	A	A	A	C	B
9. *M. pennsylvanicus* Franklin Co., MA	F	C	C	D	D	C	A	A	A	A	C	B/0.95 E/0.05
10. *M. californicus* San Bernardino Co., CA	A	C	C	C	D	C	A	A	A	A	A	B
11. *M. californicus* Sonoma Co., CA	A	C	A/0.30 C/0.70	C	C/0.15 D/0.85	C	A	A	A	A	C	B
12. *M. pinetorum* Franklin Co., MA	A	C	C	C	D	C	B	A	A	A	C	B
13. *M. pinetorum* AR	A	C	C	A/0.20 C/0.80	D/0.90 E/0.10	A/0.20 C/0.80	B	A	A/0.70 C/0.30	A	C	B
14. *M. quasiater* Veracruz, Mex.	C	C	C	C	D	C	A	A	A	A	C	B
15. *Clethrionomys* Warren Co., PA	B	B	A/0.80 C/0.20	C	B/0.20 C/0.80	C	A	A	A	A/0.40 B/0.60	B	C

Table 2.—*Coefficients of Rogers' (1972) genetic distance for all paired combinations of populations of* Microtus *and* Clethrionomys gapperi *examined.*

Species and populations	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. *M. longicaudus*	—														
2. *M. longicaudus*	0.083	—													
3. *M. mexicanus*	0.361	0.323	—												
4. *M. mexicanus*	0.396	0.361	0.088	—											
5. *M. ochrogaster*	0.238	0.196	0.302	0.307	—										
6. *M. ochrogaster*	0.202	0.161	0.270	0.256	0.063	—									
7. *M. oregoni*	0.334	0.278	0.352	0.363	0.204	0.176	—								
8. *M. montanus*	0.351	0.310	0.278	0.246	0.244	0.194	0.273	—							
9. *M. pennsylvanicus*	0.353	0.308	0.357	0.324	0.292	0.250	0.297	0.204	—						
10. *M. californicus*	0.258	0.233	0.316	0.352	0.204	0.154	0.273	0.240	0.361	—					
11. *M. californicus*	0.268	0.240	0.330	0.374	0.220	0.178	0.286	0.264	0.383	0.104	—				
12. *M. pinetorum*	0.274	0.233	0.316	0.358	0.204	0.154	0.193	0.240	0.361	0.160	0.184	—			
13. *M. pinetorum*	0.308	0.275	0.311	0.350	0.262	0.234	0.267	0.229	0.338	0.240	0.259	0.080	—		
14. *M. quasiater*	0.267	0.223	0.296	0.268	0.119	0.086	0.200	0.184	0.274	0.184	0.208	0.184	0.256	—	
15. *Clethrionomys*	0.574	0.532	0.577	0.588	0.462	0.498	0.453	0.545	0.620	0.505	0.485	0.465	0.527	0.476	—

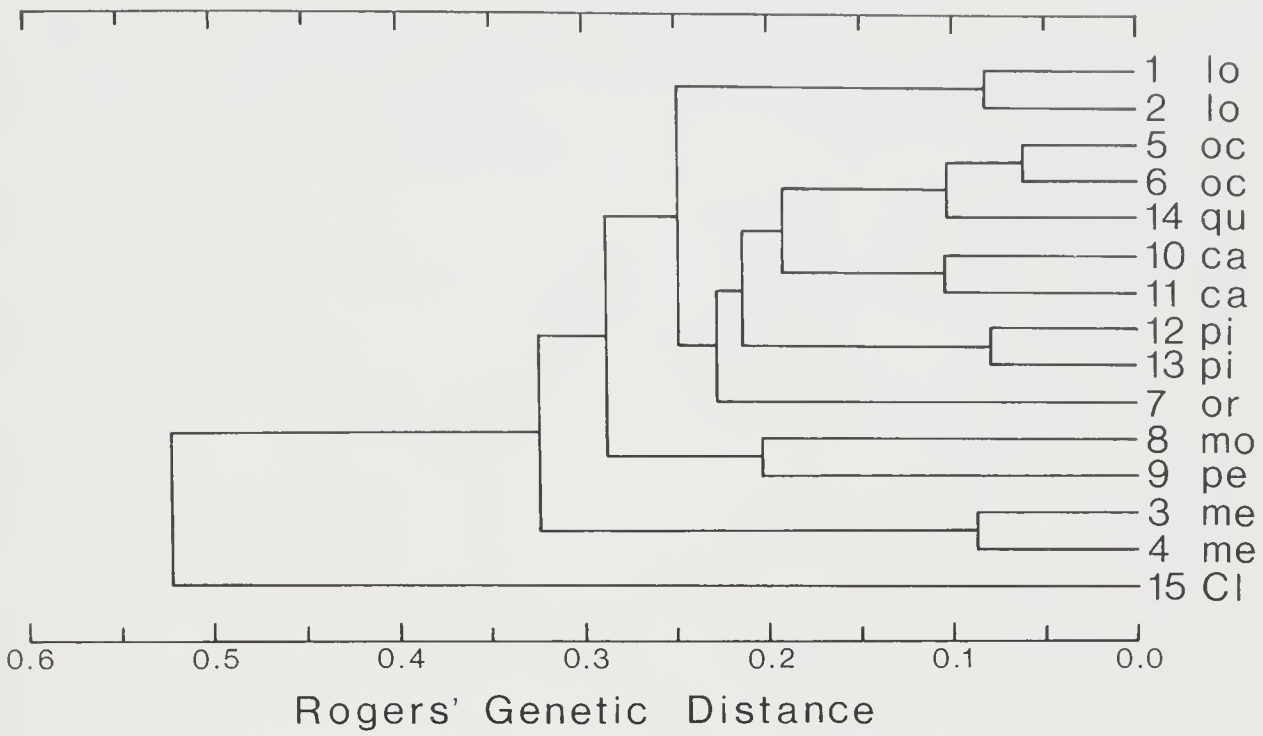

Fig. 1.—UPGMA phenogram based upon Rogers' (1972) genetic distance values for *Clethrionomys gapperi* and populations representing nine species of *Microtus* from North America. Cophenetic correlation coefficient = 0.93. Population designations are as in Table 1. Taxa are abbreviated as follows: ca = *M. californicus,* Cl = *Clethrionomys gapperi,* lo = *M. longicaudus,* me = *M. mexicanus,* mo = *M. montanus,* oc = *M. ochrogaster,* or = *M. oregoni,* pe = *M. pennsylvanicus,* pi = *M. pinetorum,* qu = *M. quasiater.*

correspond to observed genetic distances (*D*) among populations. North American *Microtus* were separated into five groups in this unrooted tree: (1) *M. ochrogaster* was genically most similar to *M. quasiater,* (2) *M. pinetorum* was most similar to *M. californicus,* (3) *M. montanus, M. pennsylvanicus,* and *M. mexicanus* were associated with one another, (4) *M. longicaudus* occupied a separate branch, and (5) *M. oregoni* occupied a separate branch, distinct from all other species of *Microtus* examined. *C. gapperi* was the most genically divergent taxon, as indicated by its branch length in the tree.

DISCUSSION

Analyses of allozyme variation indicate that *M. oregoni* is distinct from other North American microtines examined, as indicated by the association of *M. oregoni* with *Clethrionomys gapperi* in the Fitch-Margoliash tree (Fig. 2). Other investigations have also documented the morphologic (Hooper and Hart, 1962) and karyotypic (Matthey, 1957) distinctness of *M. oregoni.* Anderson (1960) and Chaline (1974) placed *M. oregoni* in the subgenus *Chilotus,* which includes only this Nearctic species. Although the Fitch-Margoliash analysis indicates that *M. oregoni* is a lineage distinct from other North American *Microtus,* the phenogram based upon genetic distance (Fig. 1) does not corroborate the genic distinctness of this taxon. Therefore, until additional subgenera of *Microtus* can be examined electrophoretically, we follow Hooper and Hart (1962), who recognized *M. oregoni* as a distinct lineage within the subgenus *Microtus.*

Microtus longicaudus is relatively distinct genically from other North American *Microtus,* as evidenced by the allocation of *M. longicaudus* to a separate branch

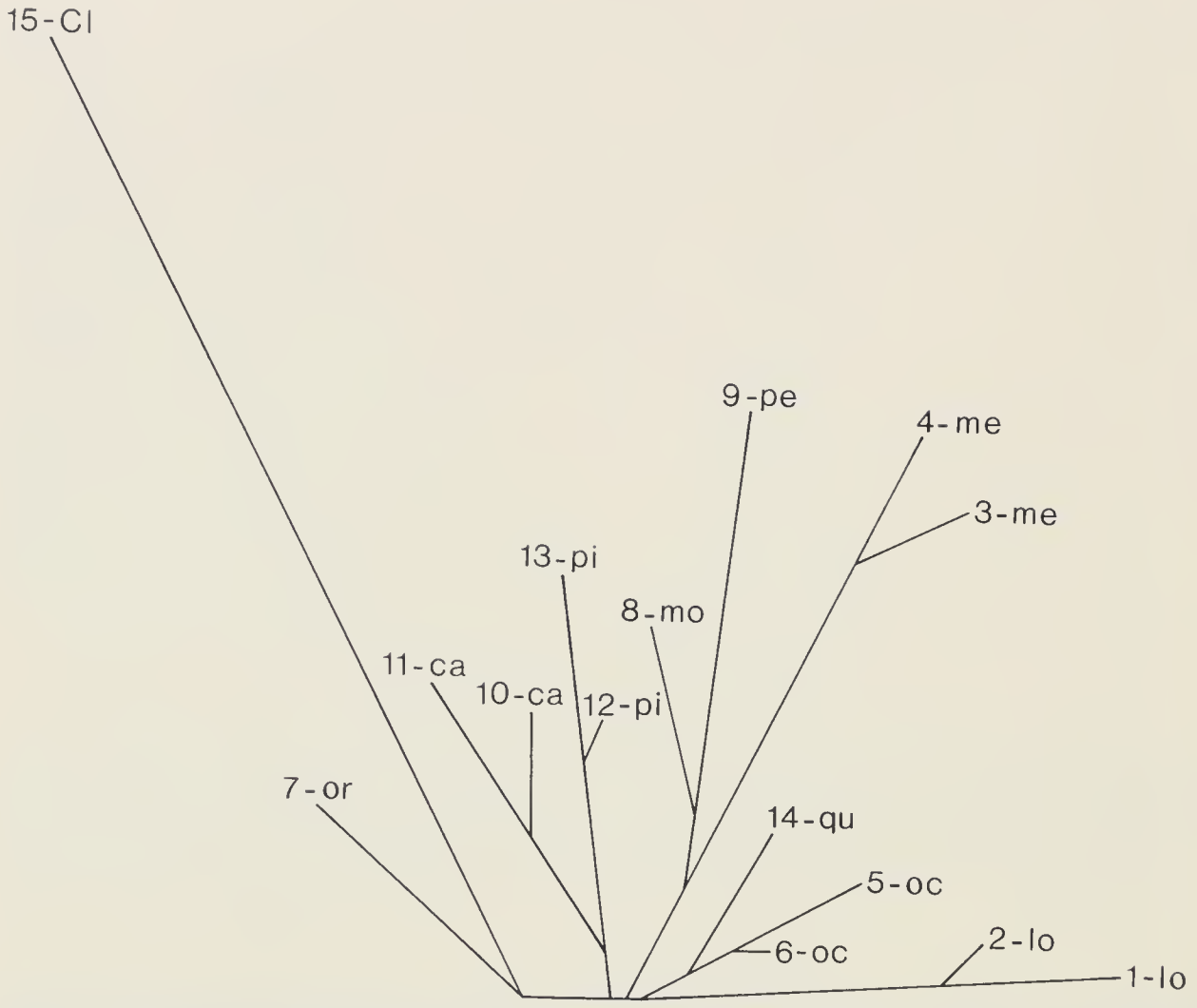

Fig. 2.—Unrooted tree based upon Fitch and Margoliash (1967) analysis of Rogers' (1972) genetic distance values among *Clethrionomys gapperi* and populations representing nine species of *Microtus* from North America. Average percent standard deviation (Fitch and Margoliash, 1967) = 7.32. Population designations are as in Table 1. Taxa are abbreviated as follows: ca = *M. californicus,* Cl = *Clethrionomys gapperi,* lo = *M. longicaudus,* me = *M. mexicanus,* mo = *M. montanus,* oc = *M. ochrogaster,* or = *M. oregoni,* pe = *M. pennsylvanicus,* pi = *M. pinetorum,* qu = *M. quasiater.*

in the Fitch-Margoliash tree (Fig. 2). This conclusion was corroborated by Modi (1987), who determined *M. longicaudus* to be chromosomally very distinct from other North American microtines. Genic analyses of Graf (1982) and analyses of the glans penis (Hooper and Musser, 1964) also indicated that *M. longicaudus* was distinct from other North American species of *Microtus.*

Allozyme analyses indicate that *M. pennsylvanicus* and *M. montanus* are most similar to one another. These taxa are associated with one another in the phenogram (Fig. 1) and in the Fitch-Margoliash tree (Fig. 2). Our results are corroborated by the chromosomal banding study of Modi (1987) and analyses of the glans penis (Hooper and Musser, 1964), in which *M. pennsylvanicus* and *M. montanus* were determined to be closely related taxa. The Fitch and Margoliash (1967) analysis (Fig. 2) also associated *M. mexicanus* with *M. pennsylvanicus* and *M. montanus.* Although Modi (1987) found the karyotype of *M. mexicanus* to be too primitive to accurately assess the taxonomic affiliation of this taxon, Chaline (1980) found *M. mexicanus* to be related to *M. montanus,* based upon analyses of dental characters.

Repenning (1983) split the nominal taxon *Pitymys pinetorum* into two species based upon morphologic criteria: *P. pinetorum* of the deciduous woodlands of the eastern United States, and *P. nemoralis* of the drier grassland habitats of the east-central United States. Repenning (1983) considered *P. nemoralis* to be more closely related to the nominal taxon *P. quasiater* of Mexico and to European species of *Pitymys* than it was to *P. pinetorum.* The apparent division of *M. pinetorum* into two taxa has additional support from observations of differences in chromosome fundamental number between eastern and western populations (Modi, 1987; Wilson, 1984). Our electrophoretic sample of *M. pinetorum* included individuals from Massachusetts and Arkansas, representing the taxa *P. pinetorum* and *P. nemoralis,* respectively, of Repenning (1983). There was a fixed electrophoretic difference at one locus (G6P) and a genetic distance value (Rogers, 1972) of 0.080 between the Arkansas and Massachusetts populations. However, given the large geographic distance separating the populations and the small sample sizes, one fixed difference does not lend strong support to recognition of *M. nemoralis* as a species separate from *M. pinetorum.* In addition, the Arkansas population of *M. pinetorum* did not associate genically with *M. quasiater,* as predicted by Repenning (1983). Therefore, until additional samples from throughout the range of *M. pinetorum* can be examined, we follow Hall (1981) and consider the nominal taxon *P. nemoralis* (*sensu* Repenning, 1983) to be a subspecies of *M. pinetorum.*

Repenning (1983) examined dental characters of fossil and extant microtines and reported that the nominal taxa *M. pinetorum* and *M. ochrogaster* were most closely related to one another; these taxa had changed little from fossil specimens of *Allophaiomys* from North America. Repenning (1983) advocated allocation of *M. pinetorum* and *M. ochrogaster* to the genus *Pitymys,* and specifically to the *Pitymys pinetorum* species group. The nominal taxa *P. quasiater* and *P. nemoralis* (currently a subspecies of *M. pinetorum*; Hall, 1981) were determined to be most closely related to one another and were placed by Repenning (1983) in the *P. quasiater* species group. Repenning (1983) believed these North American taxa to be valid representatives of the genus *Pitymys,* as distinct from North American species of *Microtus* as are European species of *Pitymys* from European species of *Microtus.* Repenning's (1983) conclusions were corroborated to some extent by the chromosomal banding study of Modi (1987), in which *M. ochrogaster* was placed as most closely related to *M. pinetorum.* However, Modi (1987) cautioned that the association of *M. ochrogaster* with *M. pinetorum* was based upon questionable karyotypic evidence. Genic data do not corroborate Repenning's (1983) conclusions regarding the kinship of *M. pinetorum* with *M. ochrogaster.* Our analysis of allozyme variation indicates that *M. ochrogaster* is most similar to *M. quasiater,* whereas *M. pinetorum* is most similar to *M. californicus* (Fig. 2). Although no study of North American microtines other than Repenning's (1983) has included *M. quasiater,* the association of *M. pinetorum* with *M. californicus* was corroborated by the genic analyses of Graf (1982). Our analysis of genic data leads us to conclude that *M. pinetorum* and *M. ochrogaster* are not most closely related to one another. However, allozyme data do support the association of *M. ochrogaster* with *M. quasiater,* as originally suggested by Repenning (1983), based upon analyses of dental characters.

Species have been placed within *Pitymys* based primarily upon the morphology of the molars (Hooper and Hart, 1962; Repenning, 1983; Van der Meulen, 1978; Zakrzewski, 1985). However, Anderson (1985) questioned the hypothesis of mo-

nophyly for *Pitymys*. Chaline and Graf (1988) believed that the characters used to assign Palearctic and Nearctic species to *Pitymys* were primarily shared primitive characters that do not reveal true phylogenetic relationships. Tooth enamel patterns commonly used as characters may be subject to considerable convergence due to environmental and/or selection factors and thus may not yield information concerning recentness of common ancestors. Howell (1924, 1926) pointed out that occlusal patterns are exceedingly variable within populations and that the morphology of the crown represents specializations for feeding. Guthrie (1965) found the morphology of the molars to be quite variable and postulated that this was due to rapid evolutionary change resulting from selection caused by a recent (early Pleistocene) shift in food habits to the vegetative parts of plants. On the other hand, natural selection probably played a much smaller role in the patterning of allele frequencies between species. If this is true, the phylogeny proposed for *Microtus* based upon morphology is subject to more convergence and parallelism than is one based upon genic data.

No Palearctic species assigned to *Microtus* or *Pitymys* were included in this study and thus the relationships among New World and Old World species could not be evaluated. However, Chaline and Graf (1988) and Graf and Scholl (1975) presented evidence that *Pitymys* is not a monophyletic group in the Old World, for the species assigned to *Pitymys* did not form a single cluster based upon phenetic analyses of genic data. These conclusions were supported by Graf (1982). Modi (1987) also concluded, based upon chromosome analyses, that the nominal taxon *M. pinetorum* was not distinct from North American species of *Microtus*. Our findings agree with those of Chaline and Graf (1988), Graf (1982), Graf and Scholl (1975), and Modi (1987), supporting the hypothesis that the taxon *Pitymys* does not represent a monophyletic lineage. These findings indicate that European taxa currently assigned to the genus *Pitymys* may require nomenclatoral as well as taxonomic revision, for the type-species for the genus *Pitymys* is the nominal taxon *P. pinetorum* of North America.

ACKNOWLEDGMENTS

We thank T. L. Yates of the Biology Department, University of New Mexico, for allowing us access to laboratory facilities; and G. A. Heidt, D. S. Rogers, and R. M. Warner for gifts of tissues or animals. S. Anderson, D. W. Duszynski, S. L. Gardner, R. S. Hoffmann, C. W. Kilpatrick, C. F. Nadler, D. A. Schlitter, N. J. Scott, and T. L. Yates commented on an earlier draft of this manuscript. Financial support for this study was provided by the Graduate Research Allocation Committee, Biology Department, University of New Mexico; Grants-In-Aid of Research, Sigma Xi; the Theodore Roosevelt Memorial Fund; HHS NIH Grant RR-08139 to D. W. Duszynski and T. L. Yates; and National Science Foundation Grant DEB-8004685 to T. L. Yates.

LITERATURE CITED

ANDERSON, S. 1960. The baculum in microtine rodents. University of Kansas Museum of Natural History Publication No. 12:181–216.

———. 1985. Taxonomy and systematics. Pp. 52–83, *in* Biology of New World *Microtus* (R. H. Tamarin, ed.), Special Publication, American Society of Mammalogists, No. 8, 893 pp.

CHALINE, J. 1974. Esquisse de l'evolution morphologique, biometrique et chromosomique du genre *Microtus* (Arvicolidae, Rodentia) dans le Pleistocene de l'hemisphere nord. Bulletin Societe Geologique dc France, 16:440–450.

———. 1980. Essai de filiation des campagnols et des lemmings (Arvicolidae, Rodentia) en zone Holarctique d'apres la morphologie dentaire. Palaeovertebrata: Montpellier, Memoire Jubilaire Hommage R. Lavocat, pp. 375–382.

CHALINE, J., AND J.-D. GRAF. 1988. Phylogeny of the Arvicolidae (Rodentia): biochemical and paleontological evidence. Journal of Mammalogy, 69:22–33.

DALQUEST, W. W., E. ROTH, AND F. JUDD. 1969. The mammal fauna of Schulze Cave, Edwards County, Texas. Bulletin of the Florida State Museum, 13:205–276.

FELSENSTEIN, J. 1989. PHYLIP 3.2 manual. University of California Herbarium, Berkeley, 285 pp.

FITCH, W. M., AND E. MARGOLIASH. 1967. Construction of phylogenetic trees. Science, 155:279–284.

GORMAN, G. C., AND J. RENZI, JR. 1979. Genetic distance and heterozygosity estimates in electrophoretic studies: effects of sample size. Copeia, 1979:242–249.

GRAF, J.-D. 1982. Genetique biochimique, zoogeographie et taxonomie des Arvicolidae (Mammalia, Rodentia). Revue Suisse de Zoologie, 89:749–787.

GRAF, J.-D., AND A. SCHOLL. 1975. Variation enzymatiques et relations phyletiques entre neuf especes de Microtinae (Mammalia, Rodentia). Revue Suisse de Zoologie, 82:681–687.

GUTHRIE, R. D. 1965. Variability in characters undergoing rapid evolution, an analysis of *Microtus* molars. Evolution, 19:214–233.

HALL, E. R. 1981. The mammals of North America. 2nd edition, John Wiley and Sons, New York, 1181 pp.

HARRIS, H., AND D. A. HOPKINSON. 1976. Handbook of enzyme electrophoresis in human genetics. American Elsevier Publishing Co., Inc., New York.

HOOPER, E. T., AND B. S. HART. 1962. A synopsis of Recent North American microtine rodents. Miscellaneous Publications of the Museum of Zoology, University of Michigan, 120:1–68.

HOOPER, E. T., AND G. G. MUSSER. 1964. The glans penis in Neotropical cricetines (Family Muridae) with comments on the classification of muroid rodents. Miscellaneous Publications of the Museum of Zoology, University of Michigan, 123:1–57.

HOWELL, A. B. 1924. Individual and age variation in *Microtus montanus yosemite*. Journal of Agricultural Research, 28:977–1016.

———. 1926. Voles of the genus *Phenacomys*. North American Fauna, 48:1–66.

KRETZOI, M. 1969. Skizze ciner Arvicoliden-Phylogenie-Stand 1969. Vertebrata Hungarica, 11:155–193.

MATTHEY, R. 1957. Cytologie comparee, systematique, et phylogenie des Microtinae (Rodentia - Muridae). Revue Suisse de Zoologie, 64:39–71.

MODI, W. S. 1987. Phylogenetic analysis of chromosomal banding patterns among the Nearctic Arvicolidae (Mammalia: Rodentia). Systematic Zoology, 36:109–136.

NADLER, C. F., N. M. ZHURKEVICH, R. S. HOFFMANN, A. I. KOZLOVSKII, L. DEUTSCH, AND C. F. NADLER, JR. 1978. Biochemical relationships of the Holarctic vole genera *Clethrionomys, Microtus,* and *Arvicola* (Rodentia: Arvicolinae). Canadian Journal of Zoology, 56:1564–1575.

REPENNING, C. A. 1983. *Pitymys meadensis* Hibbard from the Valley of Mexico and the classification of North American species of *Pitymys* (Rodentia: Cricetidae). Journal of Vertebrate Paleontology, 2:471–482.

ROGERS, J. S. 1972. Measures of genetic similarity and genetic distance. Studies in Genetics. VII. University of Texas Publications, 7213:145–153.

SELANDER, R. K., M. H. SMITH, S. Y. YANG, W. E. JOHNSON, AND G. B. GENTRY. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the oldfield mouse (*Peromyscus polionotus*). Studies in Genetics. VI. University of Texas Publications, 7103:49–90.

SWOFFORD, D. L., AND R. B. SELANDER. 1981. BIOSYS-1, a computer program for the analysis of allelic variation in genetics. Department of Genetics and Development, University of Illinois, Urbana, 65 pp.

VAN DER MEULEN, A. J. 1978. *Microtus* and *Pitymys* (Arvicolidae) from Cumberland Cave, Maryland, with a comparison of some New and Old World species. Annals of Cargenie Museum, 47:101–145.

WILSON, J. W. 1984. Chromosomal variation in pine voles, *Microtus* (*Pitymys*) *pinetorum* in the eastern United States. Canadian Journal of Genetics and Cytology, 26:496–498.

ZAKRZEWSKI, R. J. 1985. The fossil record. Pp. 1–51, *in* Biology of New World *Microtus* (R. H. Tamarin, ed.), Special Publication, American Society of Mammalogists, No. 8, 893 pp.